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The Anovular Corpus luteum in the Golden Hamster (*Mesocricetus auratus* Waterhouse) and Comparisons with the Normal Corpus luteum and Follicle of Atresia

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**The anovular corpus luteum in the golden hamster
(*Mesocricetus auratus* Waterhouse) and comparisons with
the normal corpus luteum and follicle of atresia^{1, 2}**

MARSHALL HOROWITZ

Introduction

That ovulation, rupture of the Graafian follicle with ejection of an ovum, is not a prerequisite for the formation of corpora lutea has been demonstrated by various authorities. Since their modus of development differs from those events which lead to the development of normal corpora lutea, the anovular corpora lutea have evolved. The occurrence of the anovular corpora lutea has been observed in only a small number of laboratory animals; and in these it is not a common phenomenon, occurring quite infrequently. Compounded upon this is the fact that at times the anovular corpora lutea are impossible to differentiate from normal corpora lutea, the existence of the former having to be inferred on the basis of the morphology of the structure under observation. As is necessary for the detailed structure and the morphologic alterations occurring during the existence of corpora lutea and atretic follicles, exacting observation with the aid of a multiple alternate staining technique is also demanded in order that a corpus luteum be classified as of the anovular type. Although anovular corpora lutea have been observed in some laboratory animals, they have not as yet, to my knowledge, been observed in the golden hamster.

The anovular corpora lutea may occur spontaneously as observed by SOBOTTA [1896, 1897] in the mouse and rabbit, and BURKL and KELLNER [1954] in the rat. ARON [1932] not only corroborated the spontaneous origin of such corpora lutea in the rat and guinea pig, but produced them experimentally with the administration of anterior pituitary hormone; he designated the structures as “Pseudo corps jaune spontané et Pseudo corps jaune expérimental”. Recently, we have also observed the presence of anovular corpora lutea in the ovaries of albino rats (Ciba strain “Ivanovas”). That the anovular corpora lutea were not at all limited to animals

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had been demonstrated by the observations on human ovaries by WALLART [1914], STIEVE [1926] and LUDWIG and HOROWITZ [1966].

Graafian follicles may be transformed into anovular corpora lutea through the experimental administration of GSH (ARON [1932]). Furthermore, this transformation is accomplished through the effects of chorionic gonadotrophin, as occurred in a patient with hydatidiform mole (WALLART [1914]). In the presence of a single Graafian follicle "whose ovum was undergoing maturation division", the uterus of an otherwise normal patient showed a "premenstrual endometrial swelling", signifying the presence of progesterone (STIEVE and MEYER [1926]). The patient had been in the 23rd day of her regularly appearing 28 day cycle, and no recent corpora lutea were present in either ovary. As a result of these observations, the anovular corpus luteum assumes endocrinological importance.

In this presentation the microscopic-anatomic structure of the anovular corpus luteum shall be described, and compared with the normal corpus luteum and the follicle of atresia in the golden hamster (*Mesocricetus auratus* Waterhouse).

Technique

Since concurrent studies were being performed, numerous golden hamsters were at our disposal. In all, the paired ovaries of seventeen (17) hamsters had been microscopically examined, the selection having been based on the expected stages of development of the ova to be recovered from the tubae uterinae and uteri. The estrous cycles of the animals had been previously determined by the daily vaginal smear technique as proposed by LUDWIG [1954]. The animals were receiving no treatment whatsoever. In ether narcosis the internal genital organs were sought out and the upper portions of the uterine horns together with the attached uterine tubes and ovaries were removed and placed in Tyrode's solution. With the aid of a binocular magnifying lens the ovaries were bluntly dissected from the rest of the genital organs, cautiously prepared from their fatty tissue investment and placed in Carnoy's solution. Embedding in paraffin was preceded by treatment with absolute alcohol and methylbenzoate. Paraffin blocks were then serially cut with the microtome, each preparation measuring $7\ \mu$ in thickness. The preparations were alternately stained by the following methods: Azan, Gomori-silver impregnation for the demonstration of argyrophilic and collagenous fibers, PAS-reaction, methylgreen-pyronin and Bauer's glycogen reaction.

In the above mentioned concurrent studies the number of ova recovered from the tubae uterinae and the uteri, and their stages of development had been visualised. These ova were subsequently examined for their content on, and the distribution of various enzymes.

Observations

I. Anovular corpora lutea

The anovular corpora lutea were limited to two animals, being designated hamster I and hamster II. In the former, five (5) such corpora lutea were present in one ovary and two (2) in the other. Hamster II possessed one (1) such corpus luteum in each ovary. Eight (8) ova in the 4-cell stage of development had been recovered

from the genital tract of hamster I, and six (6) ova in the fertilized 1-cell stage from hamster II.

Morphologically, the anovular corpora lutea under observation could be classified as being representative of either early or later stages during their existence.

Anovular corpus luteum; early stage

The anovular corpus luteum (figs. 1-3) was observed in the ovary of hamster II.

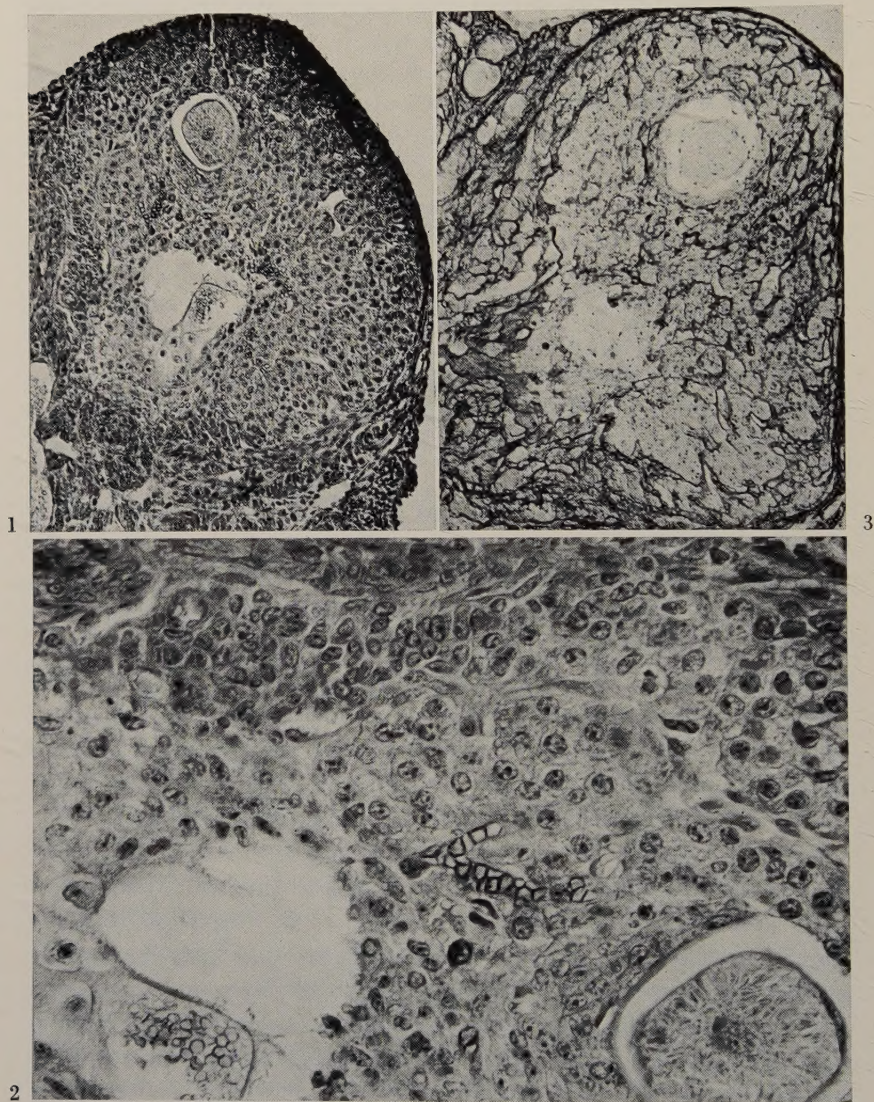
Capsule: The structure is small, in size comparable to a Graafian follicle. It is spherical in shape with one-half its circumference projecting above the surface of the ovary. The remaining portion, as well as the free area, is enclosed in a capsule which sharply demarcates the structure from its surroundings. This capsule consists of concentric interweaving strands of argyrophilic and collagenous fibers, ground substance which is weakly PAS-positive and several rows of elongated spindle-shaped cells. The nuclei of the latter are large, elliptical and clear, the cytoplasm being homogeneously clear. Metachromasia is not demonstrable. Interspersed between the fibers and the connective tissue cells are blood-filled dilated capillaries. Examination of the serial sections did not reveal evidence of an ovulation site.

Basement membrane: A remnant of the basement membrane is clearly visible. It does not extend much further than what can be seen in this section. It is thickened, wavy and stains blue with Azan. Although in the process of degeneration, the physical characteristic as a limiting membrane is still achieved. External to the basement membrane, cells of the theca interna are easily demonstrable, while just inside the membrane a small area of non-luteinised granulosa cells can be distinguished. This ever-present remainder of the membrana granulosa is in the process of degeneration, characterised by the loss of staining ability, cell-ballooning and disintegration.

Granulosa-lutein and theca-lutein cells: Granulosa-lutein cells make up the major portion of the anovular corpus luteum. Arranged in large broad trabeculae, they are enveloped by argyrophilic fibers which have extended into the body of the structure from the capsule and basement membrane. The degree of subdivision and separation of the trabeculae by intervening sinusoids is minimal, as are argyrophilisation and vascularisation. The granulosa-lutein cells are large and round to polygonal in form with distinguishable cell borders.

Their nuclei are clearly visible; they are large, round and coarsely punctuated with chromatin which stains blue-green with methylgreen-pyronin. The nucleoli are large and stain red with methylgreen-pyronin. The cytoplasm of the cells is, for the most part, evenly granulated, although some of the cells give evidence of possessing small vacuoles.

Theca-lutein cells can be seen in the peripheral subcapsular portions



of the corpus luteum. They are arranged in small groups and invested with argyrophilic fibers. These cells are of the same size as the granulosa-lutein cells. Their nuclei are large and vary in shape, from round to elliptical. They are very clear except for the nucleoli which stain red with methylgreen-pyronin. Neither the theca-lutein nor the granulosa-lutein cells exhibit metachromasia when stained with methylgreen-pyronin.

Cavum folliculi: The eccentrically situated clear area is the extent of the cavum folliculi. In it are the sequestered and degenerating granulosa cells of the partially existent membrana granulosa, and follicular fluid. The cavity is lined with a single layer of connective tissue cells and there are no fibrous elements in the cavity proper. A small circumscribed area of red blood cells, which have excluded from a capillary lying adjacent to the cavity, can be seen in the peripheral area of the follicular cavity.

Ovum: The ovum is eccentric in position. The nucleus is visible, however pycnotic, and the chromatin is clumped. The nucleoli are round and stain red with Azan. Characteristic are the rust color and the lamellar arrangement of the cytoplasm. A circumscribed area in the cytoplasm stains bright violet with PAS and with Bauer's reagent (glycogen). The membrana pellucida is broad, stains blue with Azan and pink with PAS and Bauer's reagent. The clear area surrounding the ovum can be attributed to an actual decrease in the size of the degenerative ovum and to the shrinkage effect due to fixation. A single layer of spindle-shaped connective tissue cells lines the outer perimeter of the space.

Fig. 1. Golden Hamster II (H-41). Anovular corpus luteum; early stage. Carnoy; Azan. $\times 160$.

Superficial position of corpus luteum. Degenerating ovum with pycnotic nucleus. Extravasation of blood into unorganised cavum folliculi. Presence of basement membrane in left lower quadrant. Vascularisation is moderate.

Fig. 2. Golden Hamster II (H-41). Anovular corpus luteum; early stage. Carnoy; Azan. $\times 400$.

Detailed magnification of fig. 1. Ovum with lamellar cytoplasmatic pattern. Organisation of membrana pellucida. Degenerating granulosa cells and normal theca interna cells on either side of the basement membrane. Granulosa-lutein cells between ovum and cavum folliculi.

Fig. 3. Golden Hamster II (H-41). Anovular corpus luteum; early stage. Carnoy; Gomori. $\times 200$.

Outline of ovum surrounded by broad sheets of granulosa-lutein cells. Argyrophilisation is minimal. Unorganised cavum folliculi.

Anovular corpus luteum; late stage

The anovular corpus luteum (figs. 4-6) was observed in the ovary of hamster I.

Capsule: This later stage anovular corpus luteum is larger than the foregoing. It is spherical in shape, and a small portion of it is exposed above the surface of the ovary. A connective tissue capsule envelops the structure, sharply delineating it from its surroundings. It consists of concentric interweaving strands of argyrophilic and collagenous fibers, ground substance which stains very weakly with PAS, and several rows of elongated spindle-shaped cells. Their nuclei are large, elliptical and clear, the cytoplasm of some of them being vacuolated and staining well with PAS, otherwise clear. With methylgreen-pyronin these vacuolated cells exhibit metachromasia. Interspersed between the fibers and the connective tissue cells are blood-filled dilated capillaries. The argyrophilic fibers branch from the capsule and extend into the body of the corpus luteum. They accompany the blood vessels and envelop individual and small groups of granulosa-lutein and theca-lutein cells. An ovulation site is not demonstrable.

Basement membrane and cavum folliculi: Neither the basement membrane nor the follicular cavity is present in this late stage anovular corpus luteum.

Granulosa-lutein and theca-lutein cells: Striking is the hemorrhagic area surrounding the ovum with extension almost to the capsule. Therein contained are red blood cells, fading granulosa-lutein cells, and branched spindle-shaped connective tissue cells in which PAS-reactive granulations and positive Bauer's reaction can be demonstrated. Vascular channels can be traced from this area to the periphery of the corpus luteum. When stained with the Gomori method, the area of hemorrhage exhibits an intense dense network of argyrophilic and collagenous fibers. Indistinct cellular and nuclear outlines complete the picture of granulation tissue and organisation. Between the area of hemorrhage and the capsule cords of normal and degenerating granulosa-lutein cells can be seen. The former are large, and round to polygonal in form with distinguishable cell borders. The nuclei are clearly visible; they are large, round and coarsely punctuated with chromatin which stains blue-green with methylgreen-pyronin.

The nucleoli are large and stain red with methylgreen-pyronin. The cytoplasm of the cells is, for the most part, evenly granulated, al-

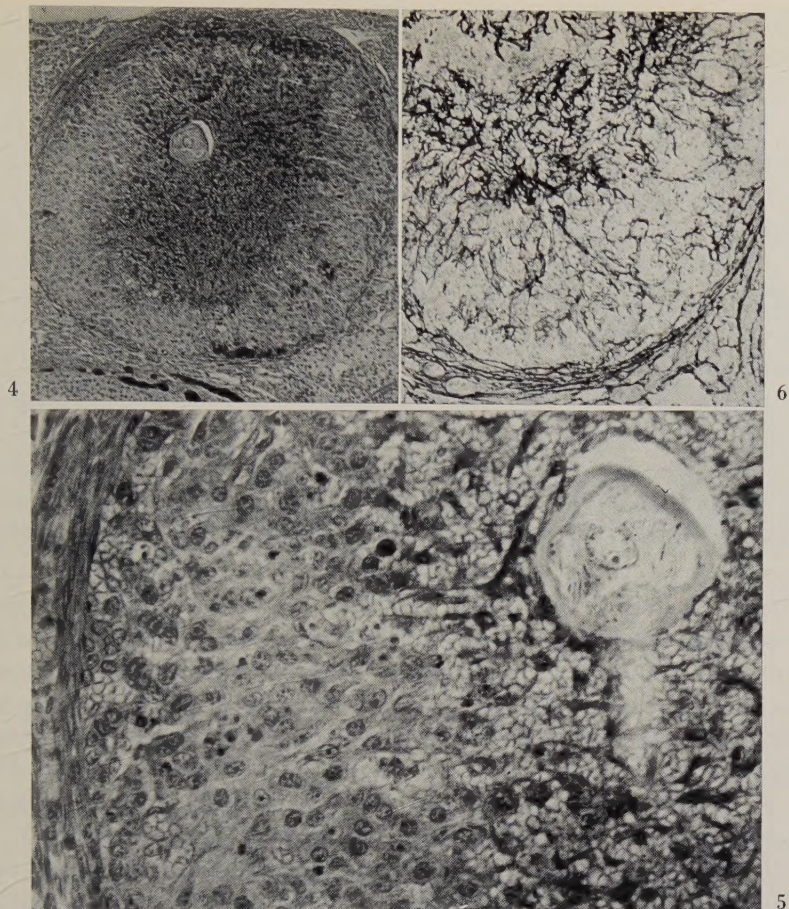


Fig. 4. Golden Hamster I (H-48). Anovular corpus luteum; late stage. Carnoy; Azan. $\times 100$.

Superficial position of corpus luteum. Slightly eccentric ovum surrounded by hemorrhagic area. Between the latter and capsule granulosa- and theca-lutein cells.

Fig. 5. Golden Hamster I (H-48). Anovular corpus luteum; late stage. Carnoy; Azan. $\times 320$.

Detailed magnification of fig. 4. Ovum with lamellar cytoplasmatic pattern, enlarged nucleoli and swollen membrana pellucida. Hemorrhage and organisation surrounding ovum. Granulosa-lutein and subcapsular theca-lutein cells.

Fig. 6. Golden Hamster I (H-48). Anovular corpus luteum; late stage. Carnoy; Gomori. $\times 200$.

Organisation with intense argyrophilic network in center of corpus luteum. Granulosa-lutein and subcapsular theca-lutein cells.

though some of the cells give evidence of possessing small vacuoles. The degenerating granulosa-lutein cells lying in the hemorrhagic area and just at its outer extension are mere shadows. With methylgreen-pyronin metachromasia can be demonstrated neither in the normal nor the degenerating cells.

Theca-lutein cells can be seen in the peripheral subcapsular portions of the corpus luteum. They, as the granulosa-lutein cells, are arranged in small groups and invested with argyrophilic fibers. These cells are of the same size as the granulosa-lutein cells and, as a result of their peripheral situation, have not as yet been affected by the hemorrhage. Their nuclei are large and vary in shape, from round to elliptical. They are very clear, except for the nucleoli which stain red with methylgreen-pyronin.

Ovum: The ovum is somewhat eccentric in position. The nucleus is clearly visible, its sap vacuolated. The nucleoli are swollen and stain blue (normally red) with Azan. Once again the rust color and lamellar arrangement characterize the cytoplasm. A circumscribed area of the cytoplasm stains bright violet with PAS and Bauer's reagent (glycogen). The membrana pellucida is extremely wide, stains blue with Azan and pink with PAS and Bauer's reagent. Lying tangential and encircling the membrana pellucida is a single layer of spindle-shaped connective tissue cells; the membrana pellucida is in the process of organisation.

Parthenogenesis: In two of the anovular corpora lutea parthenogenesis of the retained ovum was demonstrable (fig. 7).

II. Corpora lutea

Those ovaries which contained anovular corpora lutea of the type just described also possessed many normal appearing corpora lutea of varying age. On the basis of morphology alone it was possible to categorize the corpora lutea as being either fresh (early) or old (late), the estimated post-ovulation age in hours having been ascertained by the methods mentioned in the section "Technique".

Early corpus luteum

Although fresh corpora lutea were present in the animal with the early anovular corpus luteum (hamster II), the corpus luteum (figs. 8 and 9) was taken from another hamster because of its clarity. Eleven (11) ova in the fertilized 1-cell stage of development had been recover-



Fig.7. Golden Hamster II (H-41). Anovular corpus luteum; late stage. Carnoy; Azan. $\times 510$.

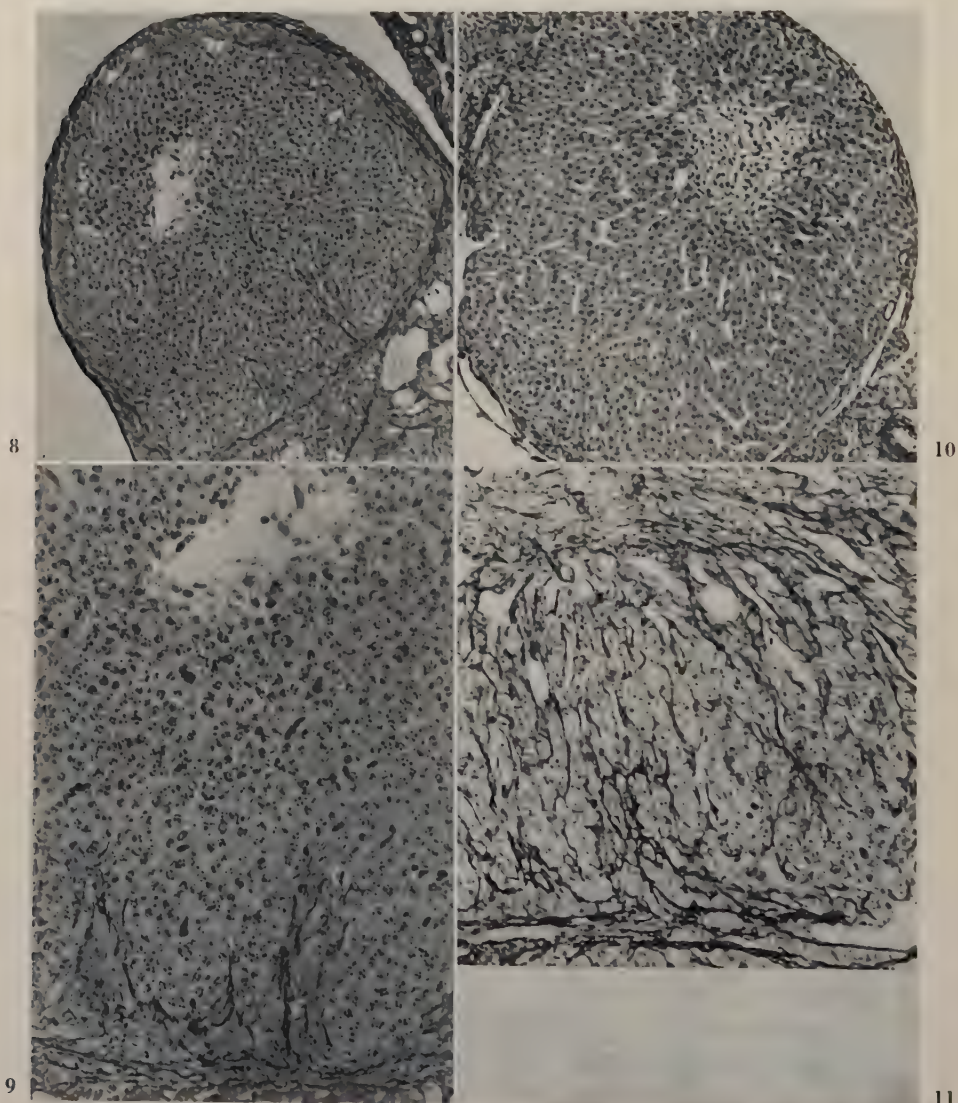
Parthenogenesis; 3 nuclei within ovum. Lamellar arrangement of cytoplasm. Hemorrhage in body of corpus luteum.

ed from the genital tract of the hamster harboring this corpus luteum. The age of this corpus luteum is estimated at approximately ten (10) hours post-ovulation.

Capsule: The structure is flask-shaped with the major portion of its circumference projecting well above the surface of the ovary. It is sharply demarcated from its surroundings by a connective tissue capsule consisting of argyrophilic and collagenous fibers, spindle-shaped connective tissue cells and blood-filled dilated capillaries. The characteristics of the capsular elements are the same as those seen and described in the anovular corpora lutea, and metachromasia cannot be demonstrated. The site of rupture (ovulation site) is demonstrable at the apical portion of the corpus luteum. The overlying mesothelium is lacking; the argyrophilic and collagenous fibers in this area are torn and project thread-like through the surface of the ovary. Slight hemorrhage with extravascular and extraovarian red blood cells can be demonstrated.

Basement membrane: The basement membrane which originally separates the membrana granulosa from the theca interna has already disappeared.

Cavum folliculi: The small central clear area is a portion of the original follicular cavity which, after ovulation has occurred, is converted into a fluid-blood-fibrin core. At a later period it will be organised by invading capillaries and young connective tissue cells. At this early stage fibrous tissue is absent from the cavity and connective tissue cells have not as yet reached its perimeter.



Granulosa-lutein and theca-lutein cells: At the periphery of the corpus luteum fine strands of argyrophilic fibers branch from the capsule, envelop groups of subcapsular theca-lutein cells and accompany the proliferating capillaries as they progress towards the center of the structure. Except for the peripheral subcapsular portion, the entire corpus luteum consists of an unbroken sheet-like mass of granulosa-lutein cells. As vascularisation progresses, this unbroken mass of cells is divided up into smaller cell groups and cords, these enveloped in argyrophilic fibers and separated from one another by intervening sinusoids. The granulosa-lutein cells are large, and round to polygonal in form. The nuclei are large, round and coarsely punctuated with chromatin; they stain pale blue-green with methylgreen-pyronin. The nucleoli stain red with methylgreen-pyronin. The theca-lutein cells are of the same size as the granulosa-lutein cells; the nuclei vary in shape, from round to elliptical, are very clear, except for the nucleoli which stain red with methylgreen-pyronin. The distribution of these cells is striking, being found subcapsular and in close contact with the proliferating capillaries. With methylgreen-pyronin the cytoplasm of the theca-lutein cells stains intensely red, while that of the granulosa-lutein cells remains homogeneously clear. Metachromasia is demonstrable neither in the granulosa-lutein nor the theca-lutein cells. Mitoses are present, although scant, in the theca-lutein cells, however, cannot be detected in the granulosa-lutein cells.

Fig. 8. Golden Hamster (H-51). Normal corpus luteum; early stage. Carnoy; Gomori. $\times 100$.

Small clear interstices at periphery indicate commencement of vascularisation. Fine strands of argyrophilic fibers and theca-lutein cells advance into granulosa-lutein cell mass towards unorganised central cavity. Ovulation site at right upper quadrant.

Fig. 9. Golden Hamster (H-51). Normal corpus luteum; early stage. Carnoy; Gomori. $\times 200$.

Detailed magnification of fig. 9. Commencement of vascularisation and migration of argyrophilic fibers and theca-lutein cells from their peripheral subcapsular position.

Fig. 10. Golden Hamster II (H-41). Normal corpus luteum; late stage. Carnoy; Azan. $\times 100$.

Radially arranged cords of granulosa-lutein cells separated from one another by vascular interstices make up major portion of corpus luteum. A small rim of theca-lutein cells is situated subcapsularly. The central cavity is completely organised.

Fig. 11. Golden Hamster II (H-41). Normal corpus luteum; late stage. Carnoy; Gomori. $\times 200$.

Detailed magnification of fig. 10. Organisation of fluid-blood-fibrin core. Total argyrophilisation of granulosa-lutein cells and groups of subcapsular theca-lutein cells.

Late corpus luteum

This later stage corpus luteum (figs. 10 and 11) was taken from the ovary of hamster II. Its estimated age is approximately fifty (50) hours post-ovulation.

Capsule: The corpus luteum is spherical in shape and is enclosed in a very thin capsule. Interspersed between the fibrous elements are spindle-shaped connective tissue cells and capillaries. That portion of the corpus luteum which projects above the surface of the ovary is covered with a one-cell layer of very flat mesothelial cells. The site of ovulation has obviously healed in this late stage.

Basement membrane and cavum folliculi: A basement membrane is not present and the follicular cavity has been completely converted into an organised fluid-blood-fibrin core.

Granulosa-lutein and theca-lutein cells: The cells making up the major portion of the corpus luteum are arranged radially in cords and trabeculae; these are the granulosa-lutein cells, having the same characteristics as those described in the earlier corpus luteum. Theca-lutein cells can be demonstrated in the subcapsular portions of the corpus luteum. The cytoplasm of these cells no longer exhibits the red granulations when stained with methylgreen-pyronin. There is no evidence of mitoses, and metachromasia of the granulosa-lutein and the theca-lutein cells cannot be demonstrated. The stage of vascularisation is complete; between the cords of granulosa-lutein cells sinusoids can be distinguished. Argyrophilic fibers envelop the radially arranged groups of granulosa-lutein cells, the theca-lutein cells and line the capillaries and sinusoids. The latter are also lined with star-shaped endothelial cells whose cytoplasm contains intensive red granulations when stained with methylgreen-pyronin. As one follows these sinusoids peripherally, they become larger, and are in direct communication with the capsular capillaries. The central area is the region of the fluid-blood-fibrin core which is in the process of organisation. Argyrophilic and collagenous fibers, capillaries, some fluid and young branched connective tissue cells can be distinguished.

III. Follicular atresia

The morphologic alterations of follicular atresia are manifold and, for the most part, constant. Figs. 12-17 demonstrate the various degenerative processes occurring in such follicles.

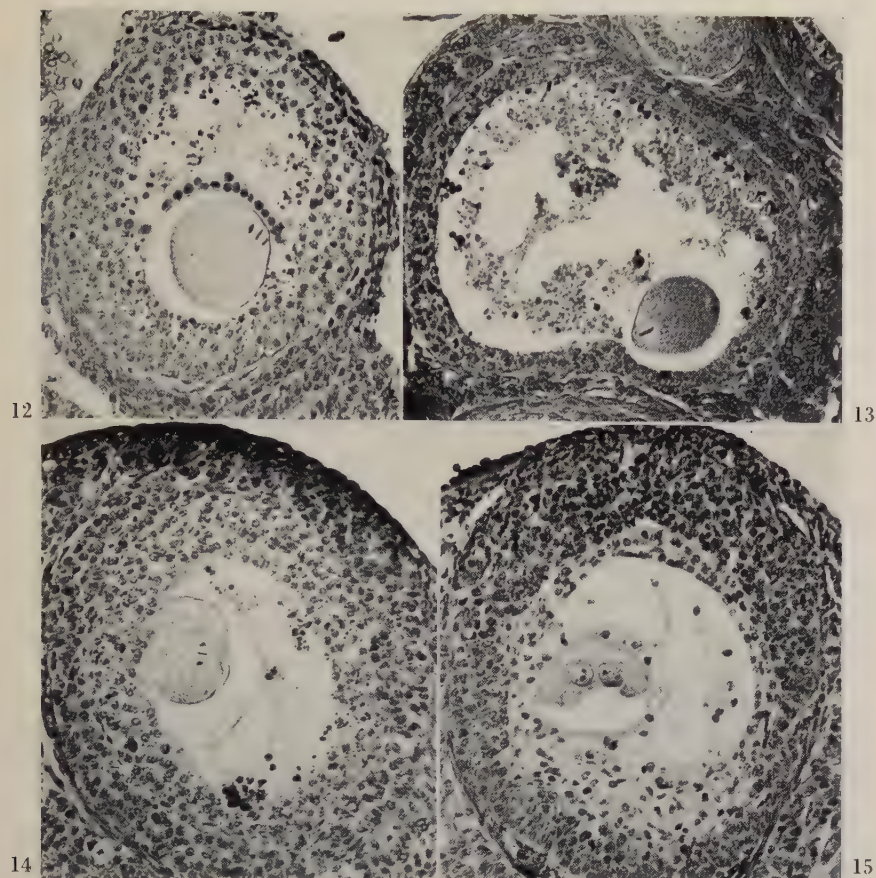


Fig. 12. Golden Hamster (H-43). Atretic follicle. Carnoy; Azan. $\times 200$.

Initial degeneration of membrana granulosa with a remnant of the corona radiata. Sequestration with cellular debris in cavum folliculi. No thecal overgrowth. Parthenogenetic mitotic spindle in anaphase with intermediate body.

Fig. 13. Golden Hamster (H-56). Atretic follicle. Carnoy; Azan. $\times 220$.

Almost complete degeneration of membrana granulosa. Sequestration of cells, cellular debris and cell shadows in cavum folliculi. No thecal overgrowth. Parthenogenetic mitotic spindle in metaphase. Cytoplasmatic clumping in ovum.

Fig. 14. Golden Hamster (H-51). Atretic follicle. Carnoy; Azan. $\times 200$.

Degeneration of membrana granulosa with cellular clumping and sequestration into cavum folliculi. Initiation of thecal overgrowth. Parthenogenetic mitotic spindle in telophase with intermediate body. Beginning of cell division with possible polar body.

Fig. 15. Golden Hamster II (H-41). Atretic follicle. Carnoy; Azan. $\times 200$.

Almost total degeneration of membrana granulosa. Connective tissue cells in cavum folliculi. Pronounced thecal overgrowth. Shrunken binucleate ovum.

Ovum: The fine granulated texture of the cytoplasm of the normal growing and mature ovum is converted into groups of parallel arranged and rust colored (Azan) lamellae. A small area of the cytoplasm stains deep violet with PAS and Bauer's reagent. The nucleus may show no demonstrable alterations, however, more commonly exhibits various stages of karyorrhexis. The nuclear sap becomes vacuolated, sometimes to a degree that the nucleus is completely clear. The nucleoli swell to approximately two to three times their original size. The chromatin becomes arranged in clumps. The staining ability of these two structures, nucleoli and chromatin, becomes altered; usually staining red with Azan, they now assume a blue color. The membrana pellucida becomes extremely broadened, stains blue with Azan and pink with PAS and Bauer's reagent. As the ovum decreases in size, the membrana pellucida either adheres closely to it or becomes folded upon itself.

Parthenogenesis: Parthenogenesis of the degenerating ovum is a commonplace occurrence. As shown in the illustrative figures, all stages of nuclear division can be observed. As a result of the parthenogenetic division, a multinuclear ovum, multinuclear ova or follicles containing two or more ova arise.

Membrana granulosa: During the process of atresia the granulosa cells progress through a series of alterations which eventually culminates in their complete degeneration. A characteristic of the growing follicle is the numerous mitotic configurations in the membrana granulosa. During the process of atresia mitoses cease. The cells, while still forming the membrana granulosa, concurrently lose their staining ability; the cytoplasm becomes clear and vacuolated. The nucleus, at this stage, appears to be unaltered. At a later stage dissociation of the cellular compactness occurs. The cells, dissolving from the group, lie freely in the follicular fluid. On the one hand, cell-ballooning occurs, the cells swelling to approximately twice their original size, assuming the form of signet-ring cells and then disintegrating. On the other hand, they retain their original size, may even remain incorporated within the cell group comprising the membrana granulosa, however, the cytoplasm appears grey and granular; the nuclei and chromatin swell, clump and stain deep red with Azan. The cells themselves may become clumped together. Later, they become mere shadows of cells, blue-grey in color, and clear areas appear where the nuclei had previously been observed. With methylgreen-pyronin the cytoplasm of the degenerating cells assumes a foamy appearance

and exhibits metachromasia, while the nuclei are deep blue-violet in color. In both instances the disintegrating products are small, pale blue granular bodies which are dispersed throughout the follicular fluid. The membrana granulosa, as a result of the degenerative processes cited, disappears in its entirety.

Theca folliculi interna: With the exception of the primordial follicles, those secondary and Graafian follicles exhibiting atresia have characteristic alterations in the theca interna. A slight increase in the size of the cells and a marked increase in the number of cell layers can be demonstrated in the theca interna; theca hypertrophy and theca hyperplasia occur. This thecal overgrowth is more readily visualised in the secondary follicles and follicles which are in the very early stages of antrum formation. The process can best be demonstrated and followed with the aid of the Gomori stain (fig. 17). As the thecal cells proliferate centrally, the follicular cavity diminishes in size, fluid is reabsorbed and the basement membrane changes its appearance (see below). The results of this thecal overgrowth are spherical formations of varying sizes; their size is dependent upon the stage of follicular development attained before undergoing atresia. These formations are enclosed peripherally in the original outerlimiting capsule present around all follicles, and consisting of reticular and collagenous fibers and connective tissue cells. The structure itself consists exclusively of theca interna cells which are morphologically similar to the cells comprising the surrounding stroma. The theca cells within the structure are arranged in groups, these enveloped by argyrophilic fibers. They do not possess the red granulations (see early corpus luteum) when stained with methylgreen-pyronin. During the early stages of overgrowth vascularisation can be demonstrated. This diminishes as proliferation continues, few if any capillaries being demonstrable in the final product of thecal overgrowth. The basement membrane has disappeared and the granulosa cells cannot be demonstrated. During the proliferative stage of thecal overgrowth metachromasia of the thecal cells is not a characteristic feature, however, the final spherical formations do, in some instances, show signs of metachromasia.

Liquor folliculi and cavum folliculi: The fluid itself is unremarkable. Dispersed throughout it are the sequestered granulosa cells and their products of degeneration. In those follicles in which a large antrum can be demonstrated the degenerating ovum either assumes a position in contact with the degenerating membrana granulosa, or lies freely in the cavity. As atresia progresses, the fluid is reabsorbed, the

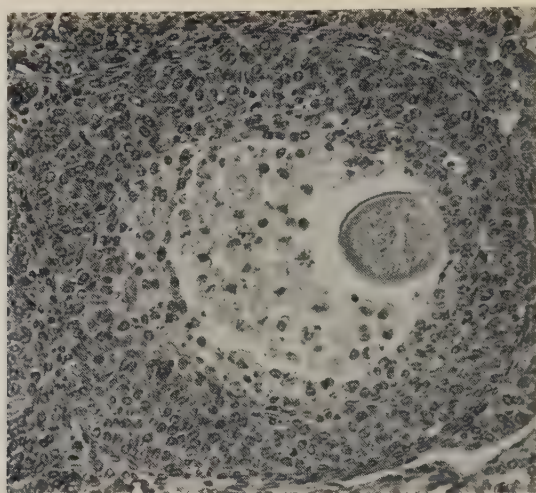


Fig. 16. Golden Hamster (H-51). Atretic follicle. Carnoy; Azan. $\times 200$. Signet-ring cell formation of membrana granulosa. Pronounced thecal overgrowth.

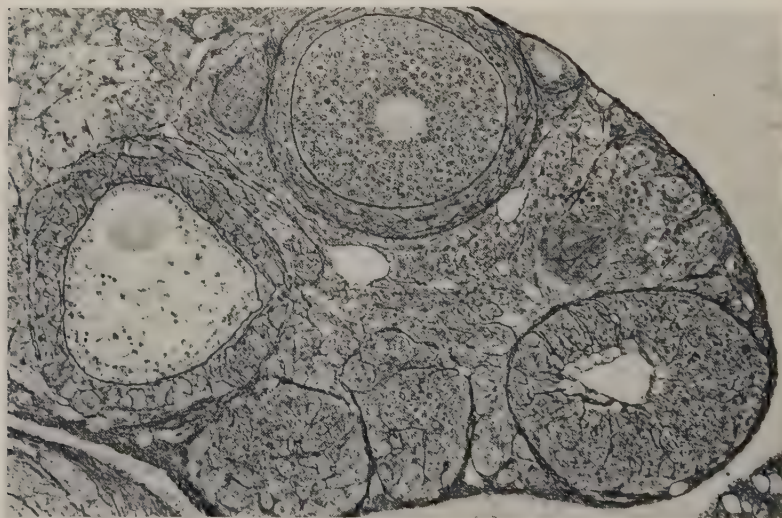


Fig. 17. Golden Hamster (H-51). Atretic and normal follicles. Carnoy; Gomori. $\times 125$. Normal follicle for comparison above. Process of atresia: follicle at left shows complete degeneration and sequestration of membrana granulosa with signet-ring cell formation and cell clumping. Degenerating ovum. Slight thecal overgrowth with wavy basement membrane. The follicle at the right demonstrates the complete disappearance of the membrana granulosa and marked thecal overgrowth with the basement membrane forming its internal limiting border. The two follicles below are the spherical end stages of thecal overgrowth. Cells are identical with those of surrounding stroma.

follicular cavity becoming smaller and finally disappearing. Thecal overgrowth aids in diminishing the size of the cavity. In the secondary follicles, by definition, there is no antrum formation.

Basement membrane: Depending upon the extent of the atretic process and the size of the follicle involved, the basement membrane exhibits various characteristics. In the large Graafian follicles, as long as a few layers of degenerating granulosa cells are still present and the follicle is expanded with follicular fluid, the membrane remains taut and little or no thecal overgrowth can be seen. As the degenerative process progresses, involving the layers of granulosa adjacent to the basement membrane, the latter gives way. The reabsorption of follicular fluid plays a very important role, as does theca hypertrophy and theca hyperplasia. The membrane becomes thickened, wavy, sometimes blurred, stains intense blue with Azan and shows little or no evidence of a positive reaction with PAS or Bauer's reagent. An actual increase in the layers of argyrophilic or collagenous fibers cannot be demonstrated. As the proliferation of the theca interna continues, the basement membrane forms its internal limiting border. With the completion of thecal overgrowth the basement membrane cannot be observed. In the smaller Graafian follicles the same alterations are more readily observed and advance at a much more rapid rate.

Outer-limiting capsule: An external capsule consisting of argyrophilic and collagenous fibers and scant spindle-shaped connective tissue cells can be seen in all follicles which are larger than primordial follicles. During the process of atresia this capsule, which delineates the follicle from the remainder of the stroma, becomes slightly thicker.

Discussion

We speak of the development of the corpus luteum with the advent of ovulation, rupture of the Graafian follicle and ejection of the ovum. However, it has been demonstrated that ovulation is not essential for the initiation of corpus luteum formation. Shortly before the turn of the century, SOBOTTA [1896, 1897] observed the occurrence of the anovular corpus luteum in the mouse and rabbit ovary. He stated that "each *ruptured* follicle is transformed into a corpus luteum even when it has not completely emptied its contents. A corpus luteum is formed which still contains an ovum". Since the follicle has ruptured, he explained the retained ovum as the sequelae of the "quickly in-

pouring blood preventing the ovum from escaping; the dehiscence of the cells of the cumulus oviger being insufficient; one was dealing with a double-ova follicle, one ovum having escaped, the second not”.

WALLART [1914], while examining the internal genital organs of a patient with hydatidiform mole, observed morphologic alterations in the ovaries from which he concluded that corpora lutea could arise from *unruptured* Graafian follicles. He had not observed a retained ovum, however, by employing the term “abortive corpora lutea”, and that they were the “transition towards atypical follicular atresia” made an attempt at classification.

As the Graafian follicle and the normal corpus luteum are structures which respond to hormonal stimulation, on the one hand, and secrete hormones, on the other, a similar relationship had become evident with respect to the anovular corpora lutea. ARON [1932] demonstrated that follicles responded to GSH administration with transformation into anovular corpora lutea (“Pseudo corps jaune expérimental”). Although no mention was made of an anovular corpus luteum, STIEVE [1926] described a single “over-ripe” Graafian follicle which, in the absence of a corpus luteum, elicited a premenstrual endometrial response of the uterine mucosa (MEYER [1926]), signifying the presence of progesterone. And lastly, the inference could be made that chorionic gonadotrophin was responsible for the alterations in the ovaries observed by WALLART [1914]. A somewhat different modus of development of the anovular corpus luteum was suggested by BURKL and KELLNER [1954]. According to their observations on the rat, the transformation of the membrana granulosa into granulosa-lutein cells originated at the cumulus oviger and then extended to involve successive portions of the granulosa until the entire epithelial membrane had been converted; a certain stimulus (a substance) from the unejected ovum supposedly elicited this transformation. It is interesting to note that although a retained ovum in a fully developed corpus luteum, hence an anovular corpus luteum, has been observed in animals, it has never been demonstrated in the human. Indirect evidence and theoretical assumptions are abundant. We, however, have recently observed one such anovular corpus luteum in a female patient, substantiating earlier hypotheses.

In essence we are dealing with a structure which develops in the absence of ovulation, morphologically and apparently functionally resembles a corpus luteum, and yet, has certain characteristics which place it in the realm of follicular atresia.

On the basis of morphology alone it is possible to categorize the normal corpora lutea as being either recent or old. And as a result of the daily vaginal smear, the day upon which sperma are found in the vagina and the stage of development of the ova recovered from the genital tract, the age (hours post-ovulation) of any corpus luteum can be estimated. This has been in complete agreement with respect to the developmental stages of the ova and the histological appearance of the ovaries, e.g. 1-cell stage ova in the genital tract with recent corpora lutea in the ovaries: blastocysts in the genital tract with old corpora lutea and no recent corpora lutea in the ovaries. By associating similar morphological stages during the existence of both the normal and the anovular corpus luteum, comparisons and conclusions can be drawn with respect to the structures under consideration in this presentation, namely the normal corpus luteum, the anovular corpus luteum and the follicle of atresia.

In the golden hamster the earliest of the corpora lutea are, for the most part, small and flask-shaped (by no means diagnostic). The smaller conical portion usually shows evidence of the ovulation site with its lacerated connective tissue, lack of overlying germinal epithelium and a coagulum consisting of red blood cells, fibrin and fluid and ejected cells from the stroma, theca interna and membrana granulosa. The outer limits of the corpus luteum are invaded by capillaries and reticular fibers; mesenchymal cells and cells of the theca interna are carried along with this process of vascularisation. The basement membrane loses its characteristic as a limiting membrane during ovulation and, as a result of the vascularisation, the fibers are apparently fragmented and to some extent incorporated into the vasculature and argyrophilisation of the lutein cells. New fibers would arise through vascularisation and proliferation of connective tissue cells of thecal origin.

In the recent corpora lutea the granulosa-lutein cells are arranged as an unbroken sheet-like mass of cells; they are approximately of the same magnitude as their precursors, the granulosa cells. SOBOTTA [1896] described the corpus luteum in the mouse as consisting exclusively of hypertrophied cells of the Graafian follicle; the cells of the theca interna did, however, increase in number, but added little to the structure of the corpus luteum. STIEVE [1943] showed that cell division did not occur in the corpus luteum, and CORNER [1945] demonstrated that the transformation of the membrana granulosa to granulosa-lutein cells succeeded without cell division. In our material

mitoses, although scant, are limited to the theca-lutein cells of the early corpus luteum; they are evident neither in the granulosa-lutein cells of the latter, nor in any of the lutein cells of the late corpus luteum. Furthermore, in the early corpus luteum the basement membrane has disappeared, vascularisation is not extensive and argyrophilisation is minimal. As further differentiation and maturation of the corpus luteum progresses, the structure as a whole increases its dimensions. The granulosa-lutein cells increase in size and are subdivided into smaller cell groups. As a result of the increased vascularisation (dilated, hyperemic capillaries and sinusoids) and, parallel to this, the increased degree of argyrophilisation, radially arranged cords and trabeculae are formed.

As compared with the granulosa-lutein cells, the theca-lutein cells are fewer in number. In the earliest of the corpora lutea they are peripheral subcapsular in position. As vascularisation progresses, the cells migrate in close contact with the advancing vasculature. In this way they are randomly distributed throughout the body of the corpus luteum, as well as retaining their original peripheral position. A quantitative difference in the theca cells of the Graafian follicles and the early and late corpora lutea can be discerned, a larger number being present in the early corpus luteum. This can be attributed to the mitotic multiplication of these cells in the early corpus luteum. However scant, they are a constant and integral constituent in the morphological configuration of the corpus luteum. Qualitative differences can also be demonstrated in the methylgreen-pyronin preparations; whereas the cytoplasm of the granulosa cells in the Graafian follicles and the corpora lutea remains unaltered, that of the theca interna cells of the Graafian follicles and the early corpora lutea shows intensive red granulations which disappear in the corpora lutea undergoing organisation of their central cavities.

Prior to ovulation, the cavum folliculi contains follicular fluid and sequestered cells of the membrana granulosa. WOLFE [1931] observed that at the time of experimentally elicited ovulation, extensive hemorrhage into the emptied cavity of the follicle only occasionally occurred. RUNGE [1923] and SCHRÖDER [1930] described hemorrhagic phenomenon at the time of menstruation; in extreme instances NOVAK [1961] designated these as corpus luteum hematomas. In our material only a negligible amount of blood has been added to the cavity as a result of ovulation. The admixture of the fluid-blood-fibrin core acts as the substrate upon which organisation occurs. As the

proliferation of vessels and connective tissue advances, alterations in the condition of this core can be traced. In the recently formed corpus luteum vessels and connective tissue approach the cavity; somewhat later, a single layer of connective tissue cells line the perimeter of the cavity, while capillaries are arranged about its circumference. Progressively, capillary sprouts penetrate into the cavity; young branched connective tissue cells and abundant collagen and reticular fibers form the granulation tissue which ultimately becomes organized and eventually obliterates the cavity.

The morphological alterations of follicular atresia have been described. During the atretic process the follicular cavity decreases in size and ultimately disappears. Many forces are involved in this process. As the ovum with its membrana pellucida undergoes degeneration, the follicular fluid is reabsorbed; however, to some extent, it may be assimilated into the homogeneous material encircling the ovum. The internal pressure within the follicle would decrease, and tension is released from the basement membrane; it becomes wavy and thicker. Since an actual increase in the amount of argyrophilic and collagenous fibers is not demonstrable, the thickening could be attributed either to impregnation and swelling with follicular fluid, simple release of tension, or a combination of both. In the large Graafian follicles, as the membrana granulosa undergoes complete degeneration and the follicular fluid is in the process of being reabsorbed, the basement membrane ultimately collapses and thecal overgrowth becomes evident. In the smaller Graafian and secondary follicles basement membrane collapse, degeneration of the membrana granulosa and thecal overgrowth occur concomitantly. It is apparent that the amount of liquor folliculi, its internal pressure effect and its rate of reabsorption are the important determinants as to the rapidity with which the cavity size diminishes and thecal overgrowth advances.

Striking is the theca-hypertrophy and the theca-hyperplasia. In the largest of the Graafian follicles the theca interna is never more than 3-5 cell layers in depth, being widest at the area of the cumulus oviger. During the process of atresia the cells enlarge only slightly, however multiply markedly. As the follicular cavity decreases in size, the theca cells, arranged in groups, invested with argyrophilic fibers and accompanied by capillaries advance centripetally, the basement membrane forming their internal border. The end result is a spherical structure consisting exclusively of theca cells which are encircled by

a fibrous capsule. The theca cell groups with their reticular fiber investments are morphologically similar to the surrounding stromal elements. In some instances metachromasia of the cells comprising these newly formed structures can be demonstrated. This theca-hypertrophy and theca-hyperplasia may be considered as being important for the following reasons: for the obliteration of the follicular cavity; for the origin of the interstitial cells; for the replacement of ovarian stroma.

With respect to the ovarian stroma in the golden hamster, a few observations seem worthy of note. In the first place, apart from the collagen and reticular fibers and a small amount of fatty tissue, the stroma consists of cells which, in all characteristics, are identical with the cells of the theca interna. The latter can be distinguished from the adjacent stroma by their concentric arrangement about the follicle and with methylgreen-pyronin. In the second place, a definitive theca externa does not exist. Lastly, scar formation and large amounts of fatty tissue can never be demonstrated; evidence of old or recent corpora albicantia et atretica with hyalinisation are not visible. For these reasons it appears possible that through thecal overgrowth the ovarian stroma is renewed.

Parthenogenesis of the ovum in atretic follicles appears to be a quite frequent phenomenon. WATZKA [1957], in his review of the literature, has shown that it is a common occurrence in animals.

According to KNIGGE and LEATHEM [1956], the interstitial cells in the ovary of the rat originated from the sequestered cells of the membrana granulosa. In our material it was evident that the granulosa underwent total degeneration. Cell ballooning with signet-ring cell formation and nuclear and chromatin clumping occurred; even the granulosa cells themselves became clumped together. In all instances the cells degenerated. The cellular debris of the degenerating granulosa cells appeared, at a later period, to become a part of the homogeneous material encircling the ovum; it consisted of the swollen folded membrana pellucida, degeneration products of the membrana granulosa and possibly products of the degenerating ovum and follicular fluid. Although a subject of much controversy, the interstitial cells appear more likely to have arisen from the cells of the theca interna than from those of the membrana granulosa as postulated by BUCHER [1965], BARGMANN [1964] and WATZKA [1964].

That the initially described anovular corpus luteum is representative of an early stage, can be demonstrated by comparing it with the

similar stage in recent normal corpus luteum. Major differences between the two are the presence of an ovum and the absence of an ovulation site in the anovular corpus luteum. Both structures are approximately the same size, which is not in accordance with the observations of BURKL and KELLNER [1954], that the anovular corpus luteum is a much smaller structure than a normal corpus luteum of corresponding age. Although having already disappeared in the latter, a remnant of the basement membrane clearly separates a small area of theca interna cells from granulosa cells in the early anovular corpus luteum. The majority of the granulosa cells show the characteristics of degeneration; however, there appears to be a gradual transition from those cells not in the process of sequestration into the granulosa-lutein cells. The granulosa-lutein cells are still arranged in broad groups. There is very little argyrophilisation and vascularisation is minimal. Theca-lutein cells are primarily peripheral subcapsular in position, few if any being dispersed within the body of the anovular corpus luteum. Both corpora lutea possess a distinct "cavum folliculi", and while the proliferation of vessels and connective tissue has not as yet reached the perimeter of the cavity in the normal corpus luteum, only a single layer of connective tissue cells line the cavity of the anovular corpus luteum. Furthermore, argyrophilic and collagenous fibers are absent from the admixture of follicular fluid and sequestered granulosa cells in the anovular corpus luteum, and from the fluid-blood-fibrin core of the normal corpus luteum. The fate of the follicular cavities appears to have some bearing with respect to the fate of the anovular corpora lutea themselves. That the cavities can be obliterated through organisation, as is the case in the normal corpora lutea, is without question. However, hemorrhage appears to be extremely frequent in the anovular corpus luteum, having occurred in all of the corpora lutea in our material. The extravasation of blood into the follicular cavity of the early anovular corpus luteum could be the commencement of a process which ultimately leads to the extremely hemorrhagic later stage anovular corpus luteum. The latter structure is much larger than its forerunner and possesses the identical constituents. The hemorrhage is extensive, in some areas extending almost to the capsule. If vascularisation were rapid, if the granulation tissue were insufficient (quantitatively and qualitatively) and if hemorrhage occurred, continuous bleeding into the cavity would result. Since these corpora lutea may be functionally active (STIEVE [1926]), it would be of

interest to know whether a local increase in the concentration of ovarian hormones would cause an increased capillary permeability as demonstrated in the uterus and vagina of the rat by HECHTER, KROHN and HARRIS [1942]. The end result would resemble a corpus luteum hematoma.

It appears evident that the morphological alterations of the Graafian follicle which ultimately lead to its transformation into an anovular corpus luteum are apparently similar with those observed in the development of the normal corpus luteum. The cellular constituents (granulosa-lutein and theca-lutein cells), their distribution, the argyrophilisation and the capillaries and sinusoids are identical. The difference and the crux of the situation is the absence of ovulation and the presence of an ovum in the anovular corpus luteum. Although a deep position of mature follicles has been accused as being a causative factor preventing ovulation, it appears to have had no effect in in our material; all of the corpora lutea are located peripherally, with a goodly portion of their circumferences projecting above the ovarian surface.

At any time during its existence, the presence of an ovum should offer very little difficulty in distinguishing the anovular corpus luteum. In the earliest stages of transformation an ovum should be present and no site of ovulation should be ascertainable. According to BURKL and KELLNER [1954], the anovular corpus luteum is a much smaller structure than a normal corpus luteum of corresponding age. This is true only in the earlier stages; according to the size of the original follicle and the degree of hemorrhage, the later stage anovular corpora lutea will be correspondingly larger. Corpora lutea at the time of ovulation and corpora lutea menstruationis occasionally show hemorrhagic tendencies, as noted above; in all of the anovular corpora lutea in our material hemorrhage was the rule. The employment of multiple alternate staining methods simplifies the recognition of such corpora lutea. One could possibly state that we were dealing with a completely luteinised follicle; an atypical follicle undergoing an atypical type of atresia. The single common denominator is the presence of a degenerating ovum. Even to the slightest detail, for example parthenogenesis, the degenerative processes are identical. However, thecal overgrowth, the presence of the basement membrane and the lack of hemorrhagic tendencies (although possible) are characteristic of the atretic follicle. Every atretic follicle appeared to rejuvenate the ovarian stroma, and not one showed signs of hemor-

rhage. And no large extravasations of blood could be observed in the absence of an anovular corpus luteum.

Summary

The microscopic-anatomic structure of the anovular corpus luteum in the golden hamster is described, and compared with the normal corpus luteum and follicle of atresia.

It appears that the morphological alterations of the Graafian follicle which ultimately lead to its transformation into an anovular corpus luteum are similar with those observed in the development of the normal corpus luteum. Since all of the anovular corpora lutea have a reasonable portion of their circumferences above the surface of the ovary, a deep position is not a causative factor inhibiting ovulation.

The major points of difference between the normal and the anovular corpus luteum are the lack of an ovulation site, the presence of an ovum and the hemorrhagic tendencies in the latter.

In follicular atresia thecal overgrowth appears important for the obliteration of the cavum folliculi, the origin of the interstitial cells and the renewal of ovarian stroma.

Résumé

Etude histologique du corps jaune anovulaire (pseudo-corps jaune) chez le Hamster doré. On en compare la structure à celle du corps jaune normal et de l'atrésie folliculaire. Il en ressort que les modifications histologiques du follicule de Graaf, aboutissant à sa transformation en un corps jaune anovulaire, sont semblables à celles que l'on observe au cours du développement du corps jaune normal. Du moment que la majeure partie de la circonférence de tous les corps jaunes anovulaires fait saillie à la surface de l'ovaire, il faut admettre que ce n'est pas la position en profondeur qui représente le facteur responsable d'une ovulation inhibée.

Les différences principales entre le corps jaune normal et le corps jaune anovulaire sont chez ce dernier: l'absence d'un point d'ovulation, la présence d'un ovule et la tendance aux hémorragies.

Dans l'atrésie folliculaire une prolifération thécale importante est responsable de l'oblitération du cavum folliculi, de la formation des cellules interstitielles et de la transformation du stroma ovarien.

Zusammenfassung

Die mikroskopisch-anatomische Struktur des anovulatorischen Corpus luteum des Goldhamsters wird beschrieben und mit denjenigen normaler Corpora lutea und atretischer Follikel verglichen.

Die morphologischen Veränderungen des Graafschen Follikels, welche schließlich zu dessen Umwandlung in ein anovulatorisches Corpus luteum führen, gleichen denjenigen, die während der Entwicklung des normalen Corpus luteum beobachtet werden. Da alle anovulatorischen Corpora lutea mit einem beträchtlichen Teil ihres Umfanges an die Oberfläche des Ovars angrenzen, kann von einer tiefen Lage als kausaler Faktor bei dem Nichtzustandekommen der Ovulation nicht die Rede sein.

Die Hauptunterschiede zwischen einem normalen und einem anovulatorischen Corpus luteum sind das Fehlen eines Sprungloches, das Vorhandensein eines Eis und die Neigung zu Hämorrhagien beim letzteren.

Das sehr starke Thecawachstum während der Follikelatresie scheint für die Obliteration der Follikelhöhle, als Ausgangspunkt für die interstitiellen Zellen und für die Erneuerung des Stroma ovarii von Bedeutung zu sein.

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